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# A New Confined Spot Test with Hydrophobic Filter Paper and Its Application to the Visual Fluorometric Determination of Trace Aluminium with 2,2'-Dihydroxyazobenzene

Emiko KANEKO, Akihiko ISHIDA, Yuji DEGUCHI, and Takao YOTSUYANAGI*
Department of Molecular Chemistry and Engineering, Faculty of Engineering,
Tohoku University, Aoba, Aramaki, Sendai 980

A new sensitive and simple spot test has been developed for the visual determination of trace element in water, based on the formation of a size confined colored ring on hydrophobic filter paper. When 0.1 $cm^3$ of aqueous solution is spotted on the water-repelling surface and evaporated in an oven, a small fleck of 6 mm diameter edged with a colored analyte is formed. This method has been successfully applied to the visual fluorometric determination of trace aluminium(III) ion with 2,2'-dihydroxyazobenzene (DHAB or $H_2L$ ). The 1:1 chelate, $[AlL]^+$, becomes localized in a concentric ring zone at pH 6.5. The detection limit is $2 \times 10^{-8}$ mol $dm^{-3}$ (0.5 ppb) by visual fluorometry with a UV lamp in the dark.

Microanalysis with a simple and sensitive method is an urgent demand for the determination of trace elements in small volume of samples. As was shown by Feigl,1) spot test with filter paper was one of the most frequently studied microanalytical methods. When a drop of the test solution is placed on filter paper or papers impregnated with reagents, the colored products can be distinctly seen in the form of characteristic flecks or rings. However, the classical methods often suffer from the lack of sensitivity and selectivity.

In this study, it was found that when a drop of an aqueous solution is placed on hydrophobic filter paper, the tiny drop retains its shape on the substrate at room temperature and a size controlled small ring is formed after evaporation, instead of rapid spreading of liquids through its coarse capillaries in conventional spot test with filter paper. A matter of great importance in the proposed method is that this substrate not only brings about a simple and effective concentration of aqueous samples but also the local enrichment of analyte in a distinct ring zone is a very great aid to the improvement of the visibility of colored or fluorescent species. As the chelating reagent, 2,2'-dihydroxyazobenzene(DHAB or $H_2L$) is used, which had been previously investigated for the determination of aluminium(III) ion by visual film colorimetry2) and by HPLC with spectrophotometric detection.3) The proposed method enables much more sensitive visual determination with the detection limit of 0.5 ppb, compared with film colorimetry, where 5 ppb of aluminium was detected.2) The sensitivity is higher than that of inductively coupled plasma emission spectrometry and comparable to graphite furnace atomic absorption spectrophotometry.

As the hydrophobic filter paper, ADVANTEC 2S from Toyo Roshi Kaisha, Ltd. was used. The reagent, DHAB, was used as received from Dojindo Laboratories and the solution (ca. $1 \times 10^{-4}$ mol $dm^{-3}$) was prepared by dissolving it in a slightly alkaline aqueous solution (pH 10) with potassium hydroxide

(3 mol dm$^{-3}$, Ultrapure) from Kanto Chemical Co. Inc. As the pH buffer solutions, 0.1 mol dm$^{-3}$ piperazine-N,N-bis (2-ethanesulfonic acid)(PIPES)-KOH (pH 6.5) and 0.1 mol dm$^{-3}$ trihydroxy(methylamino)methane - HCl (pH 8.0) were used. All other reagents used were of guaranteed reagent grade. The visual fluorometry was carried out with a TOPCON Model FI-5S UV lump with UV light of wavelength 240 - 360 nm. For the reflective fluorophotometry, a Shimadzu Model CS-9300PC flying spot scanning densitometer was used. Fluorescence and absorption measurements were made with a HITACHI Model F-2000 fluorescence spectrophotometer and a HITACHI U-3200 spectrophotometer, respectively. The spot was evaporated in a Yamato Model DK-42 constant temperature oven.

The typical procedure is as follows: an aliquot of sample solution (0.08 cm$^3$) is placed on the hydrophobic filter paper. Then, 0.01 cm$^3$ of the DHAB solution and 0.01 cm$^3$ of PIPES buffer (pH 6.5) are dropped onto the sample and dried for 30 min at 70 °C in an oven. The resulting fluorescent ring of reddish-orange color was measured by visual comparison with the standard series previously prepared under the UV light in the dark. The measurement is also carried out with the spot scanning densitometer.

The reagent, DHAB, and aluminium ion form 1:1 chelate, $[AlL]^+$, and 1:2 chelate, $[AlL_2]^-$, depending on the conditions used as reported previously.$^{2-4)}$ The acid dissociation constants of DHAB determined from the absorbance - pH curve at 20 °C and I = 0.1 ($Na_2SO_4$) are $Ka_1 = 10^{-8.20}$ and $Ka_2 = 10^{-11.6}$, respectively. The fluorescence spectra of $[AlL]^+$ in aqueous solution and in the concentric ring on the substrate are shown in Fig.1 and Fig.2 , respectively. Although the reagent, $H_2L$, is slightly fluorescent in water as shown in Fig.1, the fluorescence was not detected on the substrate. The formation of DHAB-aluminium chelate is slow at room temperature,$^{3)}$ however it goes to completion during evaporation under analytical conditions. The development of colored rings in this method was studied as a function of the initial pH conditions. The

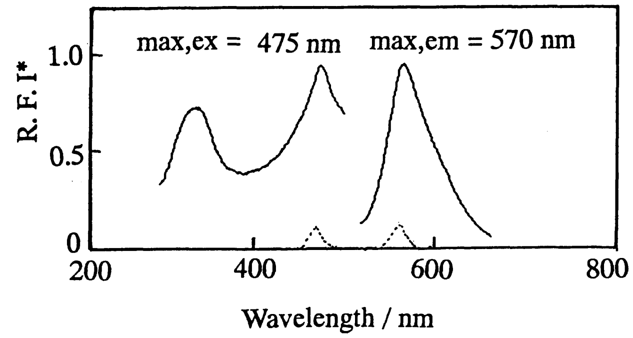

Fig.1. Fluorescence spectrum of 1:1 DHAB-aluminium chelate in aqueous solution.
——: $[Al]_T$ 1 x $10^{-6}$ mol dm$^{-3}$;
······: reagent blank; pH:6.5;
$[DHAB]_T$: 3 x $10^{-6}$ mol dm$^{-3}$;
R. F. I.* : relative fluorescence intensity to 0.01 mg dm$^{-3}$ Rhodamine B.

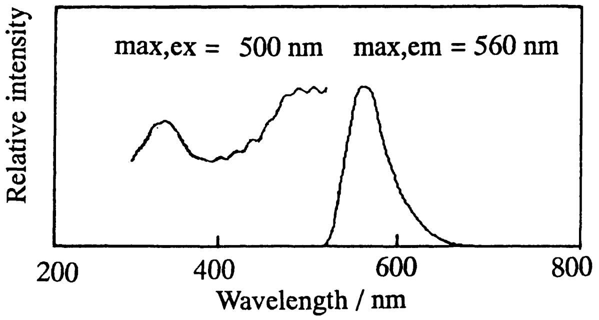

Fig.2. Fluorescence spectra of concentric ring of 1:1 DHAB-aluminium chelate.
$[Al]_T$: 1 x $10^{-6}$ mol dm$^{-3}$; $[DHAB]_T$: 3 x $10^{-6}$ mol dm$^{-3}$; pH: 6.5 (initial);
Conditions for evaporation: 70 °C, 30 min.; Substrate: hydrophobic filter paper (ADVANTEC 2S).

resulting fleck of (6.6 + 0.2) mm diameter after evaporation is edged with a fluorescent ring of the low–pH chelate, $[AlL]^+$, at pH 6.5 or with a red ring of the high–pH complex, $[AlL_2]^-$, at pH 8.0. Although the latter also visualizes aluminium ion with the sensitivity of $2 \times 10^{-7}$ mol $dm^{-3}$ at pH 8.0, it should be pointed out that the method will not work in the presence of foreign metal ions, because iron(III), copper(II), zinc(II) and other metal ions also form colored rings. Consequently, the fluorescence of $[AlL]^+$ enables the more sensitive and selective visual measurement of aluminium. The typical densitogram of the ring is shown in Fig. 3, where the fluorescence was scanned across the ring with a beam size of 0.4 x 0.4 mm. The excitation wavelength, $\lambda$ ex = 505 nm, was chosen so that the largest fluorescence intensity against the substrate was obtained. The reproducibility of the ring form assessed by the coefficient of variation of the distance between the peak tops and that of the diameter of the ring was 1.9% (mean = 4.6 mm, n = 5) and 4.3% (mean = 6.6 mm, n = 5), respectively. For the determination, scanning was carried out with a beam size of 0.4 x 10 mm to measure the fluorescent intensity of the whole ring. The coefficient of variation of the fluorescent intensity was 10.6% (n = 5) for $1 \times 10^{-6}$ mol $dm^{-3}$ of aluminium. The detection limit is $2 \times 10^{-8}$ mol $dm^{-3}$ (0.5 ppb) either with the densitometer (defined as three times the standard deviation of the blank signal ) and with visual

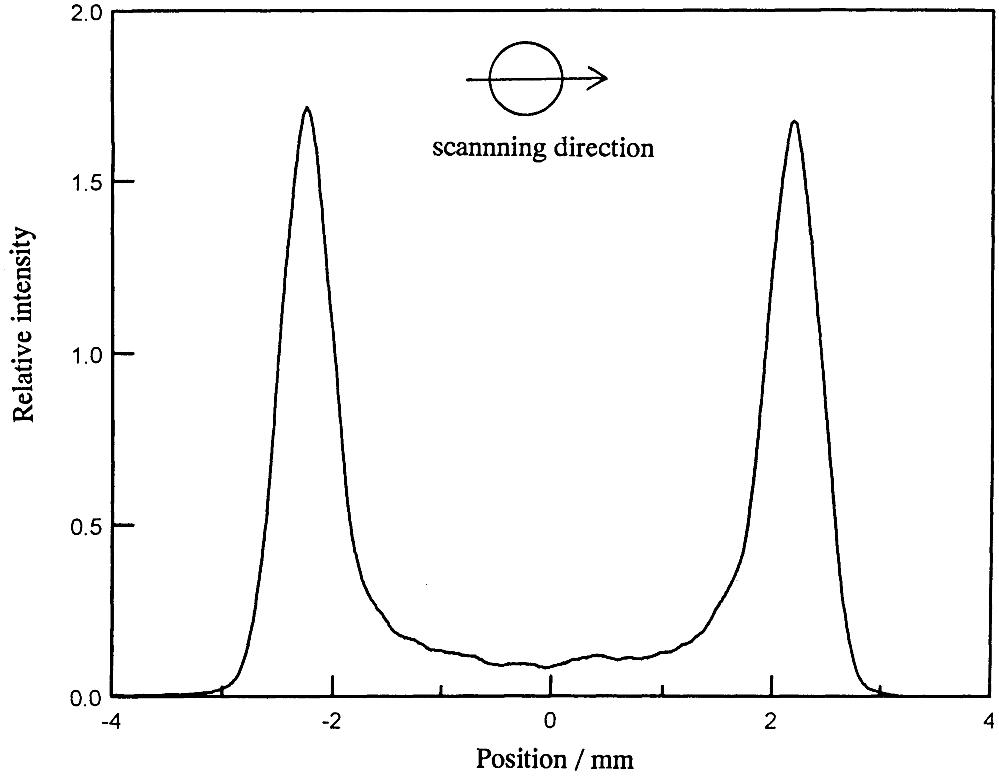

Fig.3. Typical densitogram for fluorescent ring of 1:1 DHAB–aluminium chelate.
$[Al]_T$: $1 \times 10^{-6}$ mol $dm^{-3}$; $[DHAB]_T$: $3 \times 10^{-6}$ mol $dm^{-3}$; pH: 6.5; volume of drop: 0.1 $cm^3$;
Conditions for evaporation: 70 °C, 30 min; Substrate: ADVANTEC 2S filter paper;
Conditions for measurement: $\lambda$ ex = 505 nm, beam size = 0.4 x 0.4 mm.

fluorometry. When five persons tried to detect the fluorescent rings of unknown samples with UV lamp in the dark, all of them could detect $2 \times 10^{-8}$ mol dm$^{-3}$ (0.5 ppb) of aluminium. The detection limit suggests that the sensitivity of human eye is comparable to the instruments currently available for reflective fluorophotometry. When the aluminium concentration is doubled in the range of $2 \times 10^{-8}$ to $1 \times 10^{-6}$ mol dm$^{-3}$, the intervals can be detected distinctly with naked eye. It is generally recognized that visual perception to distinguish fluorescent intensities is much more sensitive at lower concentrations.5) Therefore, the intervals of visual measurement decrease with decrease in aluminium concentrations. Interferences were studied by adding foreign ions when the test was responding to $5 \times 10^{-7}$ mol dm$^{-3}$ of aluminium ion. Copper(II), zinc(II), and magnesium(II) ions had no significant effect but $1 \times 10^{-6}$ mol dm$^{-3}$ of iron(III) ion caused the signal to decrease markedly.

Its size confining effect, which contributes so much to sensitivity, has made the hydrophobic filter paper the preferred substrate for the proposed method. However, the morphology of flecks and rings is strongly dependent upon the conditions under which it is conducted. The chelate was not concentrated in a ring zone by evaporation at room temperature and the use of 2-(N-morpholino)ethanesulfonic acid - KOH as buffer agent resulted in a decrease of the fluorescence even at the same pH (pH 6.5). The maximum sensitivity is attained only by the proper choice and establishment of the circumstances surrounding the formation of the distinct ring, i.e. substrate, buffer composition, and temperature. The basis for the alteration accompanied by evaporation to dryness via significantly high concentration of solutes still remains to be investigated.

The proposed method enables a simple and sensitive visual determination of trace element in aqueous sample of small volume. When less sample volume is available, it could be conducted with microdrops, i.e. in volumes of 0.05-0.01 cm$^3$. Further, it has been found that addition of water miscible solvent such as methanol, ethanol, and acetone to the sample solution enlarges the resulting ring and there is no or only slight decrease in sensitivity. Future work will focus on the changeful morphology and the mechanism of concentration into the ring zone to be perceived sensitively with human eye.

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